学位論文要旨（博士（理学））

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論文題名：A novel function of BAG6 in the intracellular trafficking of GLUT4.

（邦題）：GLUT4 の細胞内輸送に関わる BAG6 の新機能（英文）

GLUT4 is an essential factor for controlling blood glucose level homeostasis and is transported from the cytosolic compartment to the plasma membrane with insulin stimulation. The disfunction of GLUT4 observed in type 2 diabetes causes several serious disease complications, about nephropathy, retinopathy, and peripheral neuropathy. Therefore, it is important to elucidate the mechanism of GLUT4 translocation.

GLUT4 translocation to the plasma membrane is regulated by Rab8a, a small GTPase family protein that is critical for vesicle trafficking. Recently, we reported that BAG6 controls Rab8a stability and suggested that BAG6 has a function in Rab8a-mediated membrane trafficking. In addition, the mammalian BAG6 gene has been suggested to be linked with potential obesity- and diabetes-associated loci, while its function in the control of GLUT4 trafficking and glucose incorporation into the cytoplasm has not been investigated. Accordingly, I examined the effects of BAG6 depletion on GLUT4 trafficking.

In this study, I established a series of cell lines that stably expressed GLUT4 with three tandem repeats of the antigenic peptide inserted into its 1st extracellular loop. With these cell lines, I found that the depletion of endogenous BAG6 downregulated the cell surface expression of GLUT4, concomitant with the reduced incorporation of a glucose analog into the cells. Defective intracellular translocation of GLUT4 in BAG6-depleted cells is similar to the case observed for the depletion of Rab8a, an

essential regulator of insulin-stimulated GLUT4 translocation.

GLUT4 trafficking depends on not only Rab family protein but also SNARE family protein. SNARE protein syntaxins belong to the tail-anchored proteins (TA protein) family that is synthesized by the signal recognition particle-independent pathway. Syntaxins are required for the fusion of vesicular membranes. Previously, several reports suggested that BAG6 is a component of the transmembrane domain recognition complex and is essential for TA protein biogenesis.

During the course of my research, I noticed that BAG6 knockdown induced changes in Golgi apparatus morphology. Since the Golgi apparatus is a hub for vesicle trafficking, I hypothesized that detects in the Golgi apparatus was caused by defective biosynthesis of SNARE protein. Therefore, I examined whether BAG6 depletion leads to the misdistribution of Golgi-localized SNARE protein in the cytosol. I fractionated cell lysates by ultracentrifugation to detect cytoplasmic SNARE protein. I found that the assembly of Syntaxin 6 (Stx6) into the endoplasmic reticulum membrane was slightly disturbed under BAG6 depletion. Stx6 is a trans-Golgi-localized SNARE protein that is critical for GLUT4 translocation from the perinuclear compartment to the plasma membrane. Therefore, I examined whether BAG6 affects Stx6-dependent GLUT4 trafficking. In Stx6-depleted cells, insulin-stimulated GLUT4 translocation was decreased similar to the case for the depletion of BAG6. However, defective intracellular translocation of GLUT4 in Stx6-depleted cells was not identical to the case observed for the depletion of BAG6. Although I found that BAG6 depletion caused cytosolic/ally mis-localization of Stx6, this defect possesses only partial effects on GLUT4 Trafficking.

Given that Rab8a and Stx6 are critical for GLUT4 translocation, I propose that BAG6 may play multiple roles in the trafficking of glucose transporters to the cell surface.